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(71) Applicant *(for all designated States except US)*: **SENG ENTERPRISES LTD.** [CY/CY]; 30 Arsinoe Street,, Apartment 101, Larnaca (CY).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **DEUTSCH, Mordechai** [IL/IL]; 73 Moshav Olesh, 42855 Doar-na Lev Hasharon (IL). **DEUTSCH, Assaf** [IL/IL]; 12 Moshav Tzfaria, 60932 Moshav-tzfaria (IL).

(74) Agents: **G. E. EHRLICH (1995) LTD.** et al.; 11 Menachem Begin Street, 52 521 Ramat Gan (IL).

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(54) Title: SUBSTANTIALLY INVISIBLE THREE-DIMENSIONAL STRUCTURE FOR THE STUDY OF CELLS

(57) Abstract: A device for the study of cells comprising a structure with an index of refraction similar to that of water having a three-dimensional arrangement of fluid channels is disclosed. Also disclosed is a method for studying cells comprising seeding a structure with an index of refraction similar to that of water having a three-dimensional arrangement of fluid channels with viable cells, immersing the structure in a physiological medium, and observing the development of the seeded cells inside the structure.

SUBSTANTIALLY INVISIBLE THREE-DIMENSIONAL STRUCTURE FOR THE STUDY OF CELLS

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to the field of cellular biology and more particularly, to an improved device for the study of cells as well as a method for producing the device and methods of studying cells. The device comprises a structure having a three-dimensional arrangement of interconnected fluid channels and an index of refraction similar to that of water.

The study of cell development and behavior is an important part of many fields of science and technology including medicine, biology, pharmacology and pharmaceuticals. One important aspect of study is the effect of various stimuli or modulators such as active entities (e.g., small molecules, peptides, proteins, see below) on cell development and behavior. Another important aspect of study is that of interaction of two or more cells, that is to say, cell development and behavior in response to proximity to and the receipt of signals from another cell.

Since cell-function includes many interrelated pathways, cycles and chemical reactions and since there is a large variation of cell biochemistry amongst similar cells, the study of an aggregate of cells, whether a homogenous or a heterogeneous aggregate, does not provide sufficiently detailed or interpretable results: rather a comprehensive study of cell biological activity is advantageously performed by examining single isolated living cells as individuals. Thus, the use of single-cell assays is one of the most important tools for understanding biological systems and the influence thereupon of various stimuli such as exposure to active entities. In such studies it is important to have the ability to define multiple characteristics of each individual cell, as well as the individual cell response to the experimental intervention of interest.

In order to understand complex cellular responses to various biological modulators and other stimuli, two fundamental research capabilities are required (i) the ability to track temporal behavior of single cells as individuals for periods of minutes, hours and even days and (ii) the ability to identify and study cell heterogeneity, a phenomenon existing even in synchronized cell lines.

In the art, various different methods and devices for studying living cells are known.

Multiwell plates having 6, 12, 48, 96, 384 or even 1536 wells on a standard ca. 8.5 cm by ca. 12.5 cm footprint are well known in the art. The diameter of the wells of a plate depends on the number of wells and is generally greater than about 250 micrometers (for a 1536 well plate). The volume of the wells depends on the number of wells and the depth thereof but generally is greater than 5×10^{-6} liter (for a 1536 well plate). Although exceptionally useful for the study of large groups of cells, multiwell plates are not suitable for the study of individual cells or even small groups of cells due to the large, relative to the cellular scale, size of the wells. Cells held in such wells either float about a solution or adhere to a well surface. Developing cells clump together, making the study of the cells as individuals virtually impossible. When cells float about in a well, specific individual cells are not easily found for observation. When cells adhere to a well surface, the cells adhere to any location in the well, including anywhere on the bottom of the well and on the walls of the well. Such variability in location makes high-throughput imaging (for example for morphological studies) challenging as acquiring an individual cell and focusing thereon is extremely difficult. Such variability in location also makes high-throughput signal processing (for example, detection of light emitted by a single cell through fluorescent processes) challenging as light must be gathered from the entire area of the well, decreasing the signal to noise ratio. Further, a cell held in a well of a multiwell plate well can be physically or chemically manipulated (for example, isolation or movement of a single selected cell or single type of cell, changing media or introducing active entities) only with difficulty. Thus, multiwell plates are in general only suitable for the study of homogenous or heterogenous aggregates of cells as a group.

In the art, a number of method and devices have been developed for the study of individual cells or a small number of cells as a group. Many such methods are based on using picowell-bearing device, a device including a picowell-bearing component for study of cells. A picowell-bearing component is a component having at least one, but generally a plurality of picowells, each picowell configured to hold at least one cell. The term "picowell" is general and refers to a physical feature that localizes a cell to a specific area on a surface primarily by physical confinement and can be considered as a "small well". The term includes such physical features as wells, dimples, depressions,

pits, tubes and enclosures characterized as having a volume suitable for localizing a cell in a specific location of the picowell-bearing component, the volume defined by some physical features. Methods and devices useful for the study of cells are disclosed in PCT patent applications IL2001/00992 published as WO2003/, IL2004/000571 published as WO2004/113492, IL2004/000194 published as WO2004/077009, IL2004/000661 published as WO2005/007796, and IL2005/000801 published as WO2006/080000 which are all included by reference as if fully set forth herein.

The methods and devices discussed above provide cells with a two-dimensional environment in which to develop. Those skilled in the art are increasingly aware that the two-dimensional environment common in the laboratory is an unrealistic simulation of the natural three-dimensional environment of cells. Cell response to stimuli is usually gradient-dependent and concentration-dependent, two factors that are entirely different in two and in three dimensions. It is known that often cell metabolism and gene-expression is different in two dimensions and three dimensions, often as a result of differences in transport of oxygen, nutrients and waste products.

In the art it is known to use three-dimensional scaffolds made of gels, hydrogels and the like to define a three-dimensional environment for cell growth. Such scaffolds are not rigid so deform and vibrate, making high resolution optical study difficult if not impossible. When such layers are thick, transparency and optical inertness is compromised. Gels and hydrogels are known to be involved in or influence biological processes, for example by influencing the rate of diffusion of materials to and from cells.

It is known to use three-dimensional scaffolds made of solid material such as coral, bone or microporous foams (see for example, U.S. Patent No. 5,677,355) to define a three-dimensional environment for cell growth. Although providing cells with an environment that more accurately simulates the natural environment, individual cells can be studied only with difficulty as the cells grow inside the scaffold and are hidden from view. Since the bulk of prior art studies and methods are based on gathering optical data of cells studied in two-dimensions it is impossible to gather data that is directly comparable to data gathered from cells studied in such solid three-dimensional scaffolds.

Although in the art there are many devices for studying cells in a two-dimensional environment, in the art it is difficult to optically study cells in a three-dimensional environments, for example as a single cell developing in a volume or as a group of physically separated but interacting cells developing in a volume.

It would be highly advantageous to have a device and methods for the study of cells not having at least some of the disadvantages of the prior art.

SUMMARY OF THE INVENTION

The present invention successfully addresses the shortcomings of the art by providing a device allowing the optical study of the development of living cells in three dimensions.

According to the teachings of the present invention there is provided a device for the study of cells, comprising a structure with an index of refraction similar to that of water having a three-dimensional arrangement of interconnected fluid channels through the structure. In general, the channels define at least two (in embodiments at least ten, at least twenty, at least fifty and even at least eighty) non-coplanar interconnected fluid paths, preferably where the fluid paths start and end at an outer surface of the structure.

Typically, a device of the present invention is configured for the study of cells having a diameter of between about 5 and about 150 micrometers. Thus, in embodiments, a significant proportion (in embodiments, at least 10% by length of the total length of fluid channels through the structure) of the fluid channels have a cross-section of not more than about 160 micrometer^2 (equivalent to a 5 micrometer radius circle), that is, are small enough to prevent passage of the cells to be studied. Thus, in embodiments, a significant proportion (in embodiments, at least 10% by length of the total length of fluid channels through the structure) of the fluid channels have a cross-section of not less than about 160 micrometer^2 (equivalent to a 5 micrometer radius circle), that is, are large enough to prevent passage of the cells to be studied.

In embodiments of the present invention, a structure comprises a distribution of sizes of the fluid channels where a significant proportion of the fluid channels are large fluid channels and a significant proportion of the fluid channels are small fluid channels.

In embodiments, the structure includes a distinct population of large fluid channels and a distinct population of small fluid channels, where the average cross-sectional area of the population of small fluid channels is less than the average cross-sectional area of the population of large fluid channels.

In embodiments, large fluid channels are fluid channels configured to allow passage of cells having a diameter of at least about 5 micrometers, for example where the average cross-sectional area of the population of the large fluid channels is at least about

20 micrometers^2 (the cross sectional area of cells having a diameter of 5 micrometers). In embodiments, large fluid channels are fluid channels configured to allow passage of cells having a diameter of at least about 10 micrometers, for example where the average cross-sectional area of the population of the large fluid channels is at least about 78 micrometers^2 (the cross sectional area of cells having a diameter of 10 micrometers).

In embodiments, small fluid channels are fluid channels configured to prevent passage of cells having a diameter of at least about 3 micrometers, for example where the average cross-sectional area of the population of the large fluid channels is no more than about 3 micrometers^2 (the cross sectional area of cells having a diameter of 2 micrometers). In embodiments, small fluid channels are fluid channels configured to prevent passage of cells having a diameter of at least about 1 micrometers, for example where the average cross-sectional area of the population of the large fluid channels is at least about 0.8 micrometers^2 (the cross sectional area of cells having a diameter of 1 micrometers).

In embodiments, a structure of a device of the present invention has an index of refraction of not more than about 1.4, not more than about 1.38, not more than about 1.36, not more than about 1.35 and even not more than about 1.34. In embodiments, the structure of a device of the present invention has an index of refraction substantially equal to that of water.

In embodiments, the structure is substantially a solid. In embodiments, the structure comprises a polymer. In embodiments, the structure comprises a fluorinated hydrocarbon polymer.

In embodiments, the structure comprises an aggregate of particles of a material having an index of refraction substantially similar to that of water.

In embodiments, the structure comprises a porous block of a material having an index of refraction substantially similar to that of water. In embodiments, the structures comprises a a porous monolithic block of a material having an index of refraction substantially similar to that of water.

In embodiments, the structures comprises at least one sheet of a material having an index of refraction substantially similar to that of water. In embodiments, the at least one sheet is porous.

In embodiments, the structure comprises a stack of discrete layers of a material having an index of refraction substantially similar to that of water.

In embodiments, the structure comprises at least one filament of a material having an index of refraction substantially similar to that of water.

In embodiments, the structure is configured to fit inside a vessel for the study of cells.

In embodiments, the device comprises a porous container in which the structure is contained.

In embodiments, the device comprises a vessel in which the structure is contained, the vessel configured to hold medium (e.g., cell-growth medium). In embodiments, the structure is fixedly associated with the vessel. In embodiments, the structure is removably associated with the vessel. In embodiments, the device further comprises an aqueous solution contained in the vessel. In embodiments, the device further comprises a lid associated with the vessel configured to prevent loss of an aqueous solution from the vessel.

According to the teachings of the present invention there is also provided a method of making a structure of a device of the present invention, comprising: a) providing a plurality of porous sheets of material having an index of refraction similar to that of water; and b) stacking the porous sheets thereby making the structure. In embodiments, the sheets comprise porous membranes having an index of refraction similar to that of water. In embodiments, the sheets comprise perforated sheets. In embodiments, the sheets comprise sheets of rationally arranged filaments of material having an index of refraction similar to that of water. In embodiments, the sheets comprise sheets of woven filaments of a material having an index of refraction similar to that of water. In embodiments, the sheets comprise sheets of randomly arranged filaments of material having an index of refraction similar to that of water. In embodiments, the sheets comprise sheets of loosely associated, substantially parallel filaments material having an index of refraction similar to that of water.

According to the teachings of the present invention there is also provided a method of making a structure of a device of the present invention, comprising: a) providing at least one filament of a material having an index of refraction similar to that of water; and b) convoluting the at least one filament, thereby making the structure.

According to the teachings of the present invention there is also provided a method of making a structure of a device of the present invention, comprising: a) providing at least one sheet of a material having an index of refraction similar to that of

water; and b) arranging (including, but not limited to folding, crumpling and coiling) the at least one sheet so as to make the structure. In embodiments, the at least one the sheet of material is porous.

According to the teachings of the present invention there is also provided a method of making a structure of a device of the present invention, comprising: a) providing a plurality of particles of material having an index of refraction similar to that of water; and b) aggregating the particles so as to provide a porous block thereby making the structure. In embodiments, the aggregating includes sintering the particles.

According to the teachings of the present invention there is also provided a method for studying cells, comprising: a) seeding a structure with an index of refraction similar to that of water having a three-dimensional arrangement of interconnected fluid channels through the structure (for example, a device of the present invention) with at least one viable cell; b) immersing the structure in a medium; and c) observing development of the at least one cell inside the structure. In embodiments, 'a' precedes 'b'. In embodiments, 'b' precedes 'a'. In embodiments, the observation is optical observation. In embodiments, the method further comprises d) adding an active entity to the medium; and e) observing an effect on the at least one cell of the adding of the active entity.

As used herein, the terms “comprising” and “including” or grammatical variants thereof are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof. This term encompasses the terms "consisting of" and "consisting essentially of".

The phrase "consisting essentially of" or grammatical variants thereof when used herein are to be taken as specifying the stated features, integers, steps or components but do not preclude the addition of one or more additional features, integers, steps, components or groups thereof but only if the additional features, integers, steps, components or groups thereof do not materially alter the basic and novel characteristics of the claimed composition, device or method.

The term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners,

means, techniques and procedures by practitioners of the biological chemical, engineering, polymer and material arts.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Some embodiments of the present invention include components that are transparent or are made of a transparent material. By "transparent" is meant that the component or material is substantially transparent to at least one wavelength of light (preferably a range of wavelengths) in at least part of the visible light spectrum, the ultraviolet light spectrum and/or of infrared radiation, preferably the visible light spectrum.

The term "active entity" is understood to include chemical, biological or pharmaceutical entities including any natural or synthetic chemical or biological substance that influences a cell with which the entity is in contact. Typical active entities include but are not limited to active pharmaceutical ingredients, antibodies, antigens, biological materials, chemical materials, chromatogenic compounds, drugs, enzymes, fluorescent probes, immunogenes, indicators, ligands, nucleic acids, nutrients, peptides, physiological media, proteins, receptors, selective toxins and toxins.

The term "indicator" is understood to include any active entity that upon interaction with some stimulus produces an observable effect. In the context of the present invention, by stimulus is meant, for example, a specific second active entity (such as a molecule) released by a cell and by observable effect is meant, for example, a visible effect, for example a change in color or emission of light.

The term "medium" is understood to include a solution that allows the survival of living cells and especially nutrient media that are favorable for the growth of cells and includes, but is not limited to, such commercially available media as RPMI 1640, Fisher's, Iscove's, McCoy's, Dulbecco's Modified Eagle's Medium, etc., and the like, which may or may not be supplemented with serum, may be suitable for use as nutrient

medium. Antibiotics such as penicillin and streptomycin as well as growth factors like cytokines or hormones may also be included.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 depicts a first embodiment of the present invention, a device including a coiled sheet as a structure of the present invention;

FIG. 2 depicts a second embodiment of the present invention, a device including convoluted filaments as a structure of the present invention;

FIGS. 3A and 3B depict a third embodiment of the present invention, a device including an aggregate of particles as a structure of the present invention; and

FIG. 4 depicts a fourth embodiment of the present invention, a device including stacked sheets of woven filaments as a structure of the present invention.

In the figures herein some features may be depicted out of scale for illustrative purposes.

DESCRIPTION OF EMBODIMENTS

Embodiments of the teachings of the present invention allow the study of living cells, and especially cell development and cell communication, in three-dimensions for example using optical means. Aspects of the present invention relate to devices for the study of cells, comprising a structure having a three-dimensional arrangement of interconnected fluid channels through the structure, the structure having an index of

refraction similar to that of water. Aspects of present invention relate to methods for making embodiments of devices of the present invention. Aspects of the present invention relate to methods for the study of cells, the methods implementable using devices of the present invention.

The principles of the teachings of the present invention and the use of a device of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Device of the present invention

Generally, embodiments of the present invention are based on providing a three-dimensional structure having a three-dimensional arrangement of interconnected fluid channels through the structure, the structure having an index of refraction similar to that of water. Living cells can be held on the surface of the structure or inside the fluid channels of the structure and can subsequently develop and grow in three dimensions through the interconnected fluid channels. The interconnected channels allow the transport of nutrients and active entities to the held cells as well as the transport of waste products and secreted signaling molecules (including but not limited to hormones, growth factors, cytokines, chemokines and neurotransmitters) from the held cells. As the structure has an index of refraction that is similar to that of water, the structure is substantially invisible when immersed in an aqueous solution such as medium, e.g. cell growth medium, allowing direct optical observation of the held cells and the development thereof in three dimensions.

The three-dimensional arrangement of interconnected fluid channels is in contrast to the prior art where fluid channels are not through a structure but on a surface of a structure, and where the arrangement of fluid channels is substantially planar. In embodiments of the present invention the fluid channels define at least two (or 5, or 8 or

ten or twenty or thirty or fifty or eighty) non-coplanar (preferably substantially different) fluid paths, preferably, wherein the fluid paths start and end at an outer surface of the structure. By "fluid path" is meant a continuous path from one location of the structure to another location of the structure through the fluid channels. Preferably, substantially all of the fluid channels of a given structure are in fluid communication through the one or more fluid paths. Preferably, there are at least two, preferably many different fluid paths between substantially any two locations of the structure.

The fluid channels through a structure of the present invention are preferably such that fluid can relatively easily flow through the structure. At the same time it is generally preferred that cells seeded on or in the structure remain captured at a given location and do not migrate, but interact and grow through the interconnected fluid channels. Thus, in embodiments a significant proportion of the fluid channels is larger than or on the order of the size of cells that are to be studied but are convoluted so that cells seeded on the structure enter the bulk of the structure through a fluid channel, but are caught at some convolution. For example, in a structure configured for the study of cells that are 8 micrometers in diameter (50 micrometer^2 cross-section) a significant proportion of the fluid channels is greater than 50 micrometer^2 in cross-section. That said, in order to allow the free flow of fluids through the structure, it is advantageous that the structure includes fluid channels that are smaller than the cells to be studied, allowing fluid flow but preventing cell passage therethrough. Thus, in embodiments a structure is also provided with a significant proportion of fluid channels that are smaller than a typical cell to be studied. For example, in a structure configured for the study of cells that are 8 micrometers in diameter (50 micrometer^2 cross-section) it is advantageous that a significant proportion of the fluid channels be less than 50 micrometer^2 in cross-section. In embodiments, a structure includes two distinct populations of fluid channels: a population of "large" fluid channels that are larger than the cells to be studied allowing cell passage and growth therethrough and a population of "small" fluid channels that prevent cell passage but allow fluid flow therethrough. In embodiments, a structure includes a distribution of fluid channels that includes a significant proportion of "large" fluid channels and a significant proportion of "small" fluid channels.

Guidance as to the size, shape and other properties relating to the fluid channels through a structure of the present invention is found in the art of microporous foams used as cell growth matrices, see for example U.S. Patent No. 5,677,355.

The shape of the structure is generally any three-dimensional geometric shape. In embodiments of the present invention the shape of the structure is a member of the group comprising disks, spheres, ovoids, cones, truncated cones, pyramids (polygon base with triangular sides defining an apex), truncated pyramids, tetrahedron, prism (two congruent, parallel bases that are polygons (*e.g.*, triangle, square, pentagon, hexagon and so forth) e.g., cube, triangular prism, rectangular prism, box shaped), polyhedrons, octahedron, dodecahedron, icosahedron, cylinders, prisms and antiprisms.

In embodiments, the dimensions of a structure of the present invention are dictated in a large part by the size of the vessel in which the structure is held for use. In embodiments, the thickness of a structure is limited by factors such as the device with which cells held in the structure are to be studied, e.g. the type of microscope and the optical properties of the microscope objective used. Therefore and as discussed below, in embodiments, a structure of the present invention is no more than about 3 mm thick, no more than about 2 mm thick and even no more than about 1 mm thick.

In embodiments, a structure of the present invention is fixed. In embodiments, a structure of the present invention is deformable.

In embodiments, a structure of the present invention is substantially a porous, preferably monolithic, block. For example, in embodiments the structure comprises, essentially consists of or consists of a continuous open-cell microporous foam, analogous, for example, to the microporous foams disclosed in U.S. Patent 5, 677,355. For example, in embodiments a structure of the present invention is a frit or an aggregate of particles sintered to provide a monolithic block. One skilled in the art, for example of filtration, is able upon perusal of the description herein to select particles of the correct size, shape, size distribution and/or shape distribution so as to achieve the desired combination of "small" and "large" fluid channels. That said, in embodiments where a structure comprises an aggregate of particles, the particles making up the block are generally somewhat larger (e.g., two to five times larger) than the cells to be studied in the structure. For example, a block of sintered spheres, each sphere having a radius of about 50 micrometers is expected to define fluid channels of no less than about 18 micrometers, suitable for study of cells having diameters between about 10 and about 15 micrometers.

In embodiments, a structure of the present invention comprises a sheet (including foils and films) of material that is folded, coiled, crumpled or the like. In embodiments

where a structure is configured for the study of cells having diameters in the range of 8-30 micrometers, it is preferred to use a sheet that is up to about 20 micrometers thick. In such embodiments, the folding, coiling or crumpling generally defines "large" fluid channels. In embodiments, the sheet is porous or perforated in such a way so as to define "small" fluid channels. In such embodiments, a porous sheet is provided with pores that are typically less than about 5 micrometers in diameter.

In embodiments, a structure of the present invention comprises a plurality of stacked discrete sheets (including foils or films of material. In such embodiments, the stacking generally defines "large" fluid channels. In embodiments, the sheets are porous or perforated in such a way so as to define "small" fluid channels. The thickness of suitable sheets and the size of suitable pores is as described immediately hereinabove.

In embodiments, a structure of the present invention comprises one or more filaments (including cords, fibers, fibrils, ribbons, strips, strings, strand, thread, twine, wire, rope, yarn). In embodiments, a structure of the present invention comprises one or more filaments that are convoluted, preferably randomly, into the three-dimensional shape of the structure analogous to the convolution of a cotton ball. In such embodiments, a structure is generally not rigid but deformable. The random orientation and intersections of the one or more filaments define both "large" and "small" fluid passages. In embodiments, the structure is of substantially a single filament. In embodiments, the structure is of two or distinct more filaments. In embodiments, the two or more distinct filaments are similar in cross-sectional size and/or cross-sectional shape. In embodiments the filaments are substantially different in cross-sectional size and/or cross-sectional shape. Generally, the cross-sectional size of the filament or filaments is similar to or less than that of the cells to be studied. For example, in embodiments all dimensions of the cross section of a filament are less than about 30 micrometers, less than about 15 micrometers and even less than about 5 micrometers. In embodiments, the filaments making up a portion of a structure of the present invention are arranged in sheets, for example, woven or otherwise rationally arranged sheets so as to constitute porous sheets, that are arranged as described above, for example by stacking, folding, bending or crumpling. In embodiments, the filaments making up a portion of a structure of the present invention are randomly arranged in a sheet, for example in the manner of a non-woven cloth. In embodiments, the filament or filaments making up at least a portion

of a structure of the present invention are mutually secured at intersections, for example by partially melting or fusing.

The structure is made of any suitable material or suitable combination of materials. Preferably the structure is substantially a solid (as opposed to a gel or hydrogel) that defines the fluid channels and is not susceptible to penetration of cells. In order that the structure define a three-dimensional framework in which cells can grow and is otherwise inert and not influence or interfere with the growth of cells held therein, in embodiments a structure of the present invention is one or more of non-absorbent, non-adsorbent, non-porous, inert and non-adhesive.

As noted above, an important aspect of the teachings of the present invention is that the structure has an index of refraction that is similar to that of water. By an index of refraction similar to the index of refraction of water is meant an index of refraction of not more than about 1.4, not more than about 1.38, not more than about 1.36, not more than about 1.35 and even not more than about 1.34, or substantially identical to that of water. Such materials, once immersed in water or a medium, are virtually invisible and allow observation of cells therethrough.

One skilled in the art is acquainted with suitable materials from which to fashion a structure of the present invention, including any one or combination of a number of fluorinated hydrocarbon polymers.

A fluorinated hydrocarbon polymer suitable for implementing the teachings of the present invention is fluorinated ethylene propylene (available, for example, as Teflon® FEP from DuPont High Performance Films Circleville, OH, USA) having an index of refraction of 1.341-1.347, a density of 2.15 g ml^{-1}, is one of the most chemically inert plastics, has antistick properties, is not cytotoxic and is commercially available as a film from 12.5 micrometers and thicker, as small particles of any desired size from submicrometric diameters and as filaments (manufactured directly by extrusion or by slicing of films and sheets). A similar fluorinated ethylene propylene also suitable for fashioning a structure of the present invention is Norton® FEP fluoropolymer film available from Saint-Gobain Performance Plastics, Wayne, NJ, USA having an index of refraction of 1.341-1.347 and a density of 2.12-2.17 g ml^{-1}.

Another suitable fluorinated hydrocarbon polymer is the amorphous fluorocarbon polymer marketed under the tradename Cytop® (Asahi Glass Company, Tokyo, Japan) having an index of refraction of 1.34, a density of 2.03 g ml^{-1} which is commercially

available as a film, as small particles of any desired size from submicrometric diameters and as filaments (manufactured directly by extrusion or by slicing of films and sheets).

In embodiments, a device of the present invention is substantially entirely a structure as described above. In such embodiments, it is generally preferred that the device be configured to fit in a desired vessel for the study of cells, e.g., in a well of a 6, 12, 48, 96, 384 or 1536 multiwell plate of standard format with which one skilled in the art is familiar. In embodiments, the device is of a fixed shape. In embodiments, the device is of a non-fixed shape, e.g., is deformable.

In embodiments, a device of the present invention is contained in a porous or permeable container. In such embodiments, the porous container is configured to limit the size and shape of the associated structure for ease of handling and placing of the structure inside a vessel for use. In embodiments, the porous container is generally configured so as to not substantially interfere with the flow of fluids such as medium into and out of the structure. In such embodiments, it is generally preferred that the device, that is to say including the porous container, is configured to fit in a vessel for the study of cells, e.g., a well of a 6, 12, 48, 96, 384 or 1536 multiwell plate of standard format with which one skilled in the art is familiar.

In embodiments, a device of the present invention further comprises a vessel containing a structure of the present invention as described above. In embodiments, a structure is fixedly associated with the vessel, e.g., is integrally formed with or fixedly attached to the vessel, for example by welding or adhesive. In embodiments, a structure is removably associated with the vessel, that is can be removed from and replaced in the vessel. In embodiments, such a vessel is transparent. In embodiments, a device of the present invention substantially resembles a prior art device for the study of cells, e.g., a 6, 12, 48, 96, 384 or 1536 multiwell plate of standard format with which one skilled in the art is familiar where in one or more of the wells is disposed structure of the present invention as described above. In embodiments, a device of the present invention includes an aqueous solution or other medium, especially cell-growth medium held in the vessel, that is the structure is provided immersed in a desired medium for convenient use. In embodiments the vessel includes a heater for maintaining the temperature of a medium held in the vessel and/or an optical observation component for viewing cells in the structure.

In Figure 1 a portion of a first embodiments of the present invention, device **10**, specifically a single well **12** of device **10**, in cut out. Device **10** is substantially similar in size and shape to a prior art 1536 multiwell plate made from fluorinated ethylene propylene (Teflon® FEP, DuPont High Performance Films Circleville, OH, USA), having 1536 individual wells **12** arranged in a 32 x 48 matrix. Each well **12** of device **10** is cylindrical in shape with a radius of 0.75 mm and a depth of 3.5 mm. Well **12** is half filled with a medium **14** (RPMI 1640) that is prevented from leaking by lid **16** of 0.2 mm thick silicon rubber. Contained within well **12** is structure **18**, a 1 mm tall coiled sheet of Teflon® FEP, the coil placed so that a coiled face contacts the bottom of well **12** and is fixedly attached thereto by heat fusing.

Structure **18** is fashioned from an originally 71 mm long, 1 mm broad, 25 micrometer thick sheet of Teflon® FEP that is perforated with a plurality of pores up to about 2 micrometer holes (e.g., made mechanically or with the use of an ion beam). The sheet is embossed on both sides with a criss-cross pattern to a depth of about 7 micrometers so that the actual thickness of the sheet varies from 25 micrometers down to about 10 micrometers. The sheet is coiled, and placed inside well **12** so that the walls of well **12** hold the sheet in a coiled state where thicker sections of the sheet contact each other, holding the thinner sections of the sheet apart. In such a way, structure **18** is defined where the three-dimensional arrangement of interconnected fluid channels through structure **18** includes a population of large channels (generally from 5 to about 15 micrometers wide) defined by the distance between to layers of coil determined by the embossing and a population of small channels (up to about 2 micrometer in diameter) distinct from the population of large channels defined by the pores through the sheet. In such a way, a plurality of non-coplanar interconnected fluid paths are defined from the outer surface of structure **18** into the bulk thereof.

For use, structure **18** is seeded with cells, for example by injecting cells suspended in a medium through lid **16**. Device **10** is agitated to ensure that the cells enter the fluid channels in structure **18**. The cells are then observed from below, for example using a confocal microscope or brightfield microscope with or without deconvolution image processing software. The thickness of a structure of the present invention that can be studied, for example using a confocal microscope, is in embodiments dictated by the working distance of the objective. It is therefore preferable to use commercially available long-working distance objective lenses to study cells held in a structure of the present

invention. For example, one skilled in the art is aware that a x40 or x60 objective may have a working distance of about 2 mm. In such cases one skilled in the art may choose to study only cells held in a portion of a structure as limited by the working distance, or to provide a structure that is no more thick than the working distance of the available objective. Thus in embodiments, a structure of the present invention is no more than about 3 mm thick, no more than about 2 mm thick and even no more than about 1 mm thick.

In Figure 2 is depicted a second embodiment of the present invention, device **20**. Device **20** comprises a structure **22** of the present invention, a wad made of convoluted filaments made of fluorinated ethylene propylene (Norton® FEP) contained within a porous container **24** of an amorphous fluorocarbon polymer (Cytop® (Asahi Glass Company, Tokyo, Japan).

Structure **22** comprises a plurality of filaments each 40 mm long, 12.5 micrometers wide and 2, 5 or 10 micrometers thick. Such strands are commercially available or are easily fashioned by slicing a roll of 12.5 micrometer thick Cytop® having a 400 micrometer radius using a standard laboratory microtome to 2, 5 or 10 micrometer slices. The individual filaments are convoluted and wadded together to make a deformable wad that constitutes structure **22**. In structure **22**, the intersections of the plurality of filaments produces a distribution of randomly sized and randomly shaped fluid channels, some fluid channels large enough to allow cell passage therethrough and some so small as to prevent cell passage therethrough.

Porous container **24** is substantially a cylindrical cage containing structure **22** by the location of a plurality of struts **26**. On the periphery of container **24** are ribs **28**. Ribs **28** define a circle having the size and shape of a well of a standard 96-well multiwell plate, approximately 6 mm. Ribs **28** configure device **20** to be slidingly and removably associated with a well of a standard 96-well multiwell plate. Further, ribs **28** define an offset from the walls of a well, allowing unhinderd flow of medium into and out of structure **22**.

For use, for example, a well of a 96-well multiwell plate is partially filled with medium. A device **20** is placed in the well and a drop of medium is added, immersing device **20** and structure **22** in medium. Structure **22** is seeded with cells. The cells are then observed, for example using a confocal or brightfield microscope from below..

In Figures 3A and 3B is depicted a third embodiment of the present invention, device **30**. In Figure 3A, device **30** is depicted alone. In Figure 3B, device **30** is depicted in place inside a well of a 96-well multiwell plate. Device **30** essentially consists only of structure **32** made up of an aggregate of substantially spherical particles of an amorphous fluorocarbon polymer (Cytop® (Asahi Glass Company, Tokyo, Japan) with a size of ranging from 25 to 75 micrometers in diameter, sintered together to provide a porous monolithic block. Device **30** resembles a frit with which one skilled in the art is familiar. In structure **32**, the interstitial voids between the particles produce a distribution of randomly sized and randomly shaped fluid channels, some fluid channels large enough to allow cell passage therethrough and some so small as to prevent cell passage therethrough. The size and shape of device **30** is fixed and such that allows device **30** to be slidingly and removably associated with a well of a standard 96-well multiwell plate.

Use of a device **30** is substantially as described above and is clear to one skilled in the art upon the perusal of the description herein.

In Figure 4 is depicted a fourth embodiment of the present invention, device **34**. Device **34** essentially consists only of structure **36**, a pad made up of 50 layers, each layer a sheet of loosely woven 12.5 micrometer diameter filaments of an amorphous fluorocarbon polymer (Cytop®, Asahi Glass Company, Tokyo, Japan). In structure **36**, the voids between layers and the spaces between the filaments making up the weave produce a distribution of randomly sized and randomly shaped fluid channels, some fluid channels large enough to allow cell passage therethrough and some so small as to prevent cell passage therethrough. Structure **36** is relatively flexible but is sized so that device **34** is easily placed in and removed from a well of a standard 384-well multiwell plate.

In an embodiments, a structure **36** is manufactured by folding a single large sheet of woven filaments to achieve a stack of the desired number of layers and then cutting out the desired size and shape of structure **36** using a hot template in the fashion of a cookie cutter. In such a way the layers are fixedly associated and the weave sealed so that structure **36** maintains structural integrity.

Use of a device **30** is substantially as described above and is clear to one skilled in the art upon the perusal of the description herein.

It is important to note that in embodiments, devices of the present invention that are not fixedly associated with a vessel for the study of cells are supplied in the form of

kits, for example as a group of devices, or as a group of devices together with a suitable vessel. For example, in embodiments 96 devices **20, 30** or **34** are supplied as a kit together with a standard 96-well multiwell plate.

Method of the present invention

The method of the present invention substantially involves seeding a structure having a three-dimensional arrangement of interconnected fluid channels and an index of refraction similar to that of water (such as a device of the present invention as described above), with viable cells so that the cells are held in or on the structure, immersing the structure in a medium and observing (preferably optically) the cells held inside the structure.

In embodiments, during the study of the cells one or more active entities are added to the medium and the effect of the active entity or entities on the cells is observed.

Due to the index of refraction of the structure, the structure becomes substantially invisible, allowing optical observation of the development and growth of the cells held in the structure. As is clear to one skilled in the art, it is advantageous to observe cells held in the structure with the help of a device such as a confocal microscope so that only cells in a specific plane are in focus at any one time, allowing the unobstructed study of substantially all cells held in a device of the present invention. The structure is also ideal for observing cell using a brightfield microscope, with or without deconvolution image processing software.

In embodiments, a deformable structure is provided unassociated with a vessel for the study of cells, for example as one or more filaments. In such embodiments, the structure is placed in a desired vessel, for example by pressing inwards and compressing, folding and convoluting the one or more filaments. In such a way the structure is assembled inside the vessel.

In embodiments, a rigid structure is provided unassociated with a vessel for the study of cells, for example as a porous block of material of the size and shape of the vessel. In such embodiments, the structure is placed in a desired vessel, for example by sliding or dropping into the vessel.

In embodiments, a structure is provided contained within a porous container, for example as a convoluted filament held inside a porous container that is substantially a

cage. In such embodiments, the structure is placed in a desired vessel for the study of cells, for example by sliding or dropping into the vessel.

In embodiments, a structure is provided as an integral part of a vessel for the study of cells.

Methods of manufacture of a device of the present invention

An important aspect of a device of the present invention lies in preparation of the structure with an index of refraction similar to that of water having a three-dimensional arrangement of interconnected fluid channels through the structure as described above.

In a first step a suitable material (or materials) is chosen. Although any suitable material can be chosen, it is preferred that a material be substantially a solid (as opposed to a gel or hydrogel), for example a polymer. Exceptionally suitable polymer are fluorinated hydrocarbon polymers having a suitable index of refraction such as the fluorinated hydrocarbons available under the tradenames Teflon® FEP (DuPont High Performance Films Circleville, OH, USA), Norton® FEP (Saint-Gobain Performance Plastics, Wayne, NJ, USA) and/or Cytop® (Asahi Glass Company, Tokyo, Japan).

Such materials are commercially available as filaments of various sizes and shapes, sheets, porous sheets, particles (including submicrometeric particles), foams and sintered products. Such materials can be worked with standard techniques with which one skilled in the art is familiar including melted (e.g., for fusing, sintering or adhering), embossed, mechanically perforated, perforated with ion beams, sliced, cut and stamped.

As discussed above, in embodiments a plurality of particles of a material having an index of refraction similar to that of water are associated, for example by sintering, to provide a structure of the present invention, generally substantially a porous monolithic block or frit having a rigid shape. As noted above, one skilled in the art is able to select the size, shape, size distribution and/or shape distribution of the particles that are aggregated to provide the desired distribution and sizes of fluid channels.

As discussed above, in embodiments one or more porous sheets of material having an index of refraction similar to that of water are stacked, folded, rolled, coiled, crumpled and the like thereby making a structure of the present invention. Suitable sheets include porous sheets (e.g., permeable membranes, porous membranes), perforated sheets, sheets of woven (or otherwise rationally arranged) filament or

filaments of the suitable material, sheets of non-woven filaments of a material and sheets of loosely associated, substantially parallel filaments.

As discussed above, in embodiments of the present invention, at least one filament of a material having an index of refraction similar to that of water is convoluted thereby making the structure. In embodiments at least two filaments of a material having an index of refraction similar to that of water are convoluted together. In embodiments the at least two filaments are similar in cross-sectional size and/or cross-sectional shape. In embodiments the at least two filaments are substantially different in cross-sectional size and/or cross-sectional shape.

Some of the materials from which a structure of the present is fashioned are inherently hydrophobic. Generally, a hydrophobic structure is not considered a disadvantage. That said, in embodiments where it is undesirable that the structure be hydrophobic, but the material from which the structure is made is inherently hydrophobic, it is possible to treat the material to reduce hydrophobicity. For example, it is known to reduce the hydrophobicity of fluorinated hydrocarbon polymers by treatment with plasma.

It is expected that during the life of this patent many materials that are suitable for implementing the teachings of the present invention will be developed and the scope of the term is intended to include all such new technologies *a priori*.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above description and figures, illustrate the invention in a non limiting fashion.

Example 1: Culturing human bone marrow progenitor cells expressing the CD34 antigen.

Fibers making up a structure **22** of a device such as depicted in Figure 2 are coated with full-length fibronectin or collagen (Boehringer Mannheim, Germany) by standard incubation procedures. CD34+ haematopoietic progenitor cells derived from commercially purchased human bone marrow samples (Poietic Technologies, Gaithersburg, MD, USA) are provided. RPMI medium supplemented with 10% Fetal Bovine Serum,penicillin (10 IU/ml), streptomycin (10 ug/ml), L-glutamine (1 mM), Hepes (10 mM), Fungizone 2.5 ug/ml (Biological Industries Beit Haemek, Israel) and human recombinant cytokines; IL-6, and IL-3 at 10 ng/ml (Genzyme, Cambridge, MA, USA) is provided.

The device is immersed in the medium in a well of a standard transparent 96-well plate.

To establish a three-dimensional culture, 10 million cells/ml are seeded onto structure **22** which is agitated to distribute the cells therein.

The cell culture is incubated in a humidified atmosphere of 5% CO_2 at 37°C. The development of the cell culture is observed with the help of a confocal microscope. The medium is replaced every 2 days.

Example 2: Cell culture of mesenchymal stem cell line

Mus-musculus ATTC/CRL-12424 cells are provided. Dulbecco modified Eagle's medium (DMEM) supplemented with 4.5 g/L D-glucose, 1.5 g/L sodium bicarbonate , 1mM sodium pyruvate, 10% (v/v) fetal calf serum, 1% LGlutamin,and 1% Pen-Strep-Nystatin solution (Biological Industries, Beit Haeemek, Israel) is provided.

A device similar to the device depicted in Figure 3B is immersed in the medium in a well of a standard transparent 96-well plate.

To establish a three-dimensional culture, 6 million cells/ml are seeded onto the device which is agitated to distribute the cells therein.

The cell culture is incubated in a humidified atmosphere of 5% CO_2 at 37°C. The development of the cell culture is observed with the help of a confocal microscope. The medium is replaced every 2 days.

Example 3. Culturing primary hippocampal neurons

Primary hippocampal neurons are isolated by dissecting out Hippocampi of postnatal rats, treating for 30 minutes at 37°C with 0.25% trypsin (type XI; Sigma, St. Louis, MO), and then centrifuging. The cells are maintained in Eagle's minimal essential medium (MEM) containing 10% heat-inactivated fetal bovine serum , 2 mM glutamine, and 0.76% glucose.

A structure **36** of a device similar to that depicted in Figure 4 is coated with poly-D-lysine. The device is immersed in the medium in a well of a standard transparent 96-well plate and seeded with the neurons at a concentration of 60 million/ml. The device is agitated to distribute the cells therein.

The cell culture is incubated in a humidified atmosphere of 5% CO_2 at 37°C. The development of the cell culture is observed with the help of a confocal microscope. The medium is replaced every 2 days.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

WHAT IS CLAIMED IS:

1. A method for studying cells, comprising:

a) seeding a structure with an index of refraction similar to that of water having a three-dimensional arrangement of interconnected fluid channels through said structure with at least one viable cell;

b) immersing said structure in a medium; and

c) observing development of said at least one cell inside said structure.

2. The method of claim 1, wherein said observation is optical observation.

3. The method of claim 1, further comprising:

d) adding an active entity to said medium; and

e) observing an effect on said at least one cell of said adding of said active entity.

4. A device for the study of cells, comprising a structure with an index of refraction similar to that of water having a three-dimensional arrangement of interconnected fluid channels through said structure.

5. The device of claim 4, wherein said channels define at least two non-coplanar interconnected fluid paths, preferably, wherein said fluid paths start and end at an outer surface of said structure.

6. The device of claim 5, wherein said fluid paths start and end at an outer surface of said structure.

7. The device of claim 4, wherein a significant proportion of said fluid channels have a cross-section of not more than about 160 micrometer^2.

8. The device of claim 7, wherein said significant portion is at least 10% by length of the total length of fluid channels through said structure.

9. The device of claim 4, wherein a significant proportion of said fluid channels have a cross-section of not less than about 160 micrometer^2.

10. The device of claim 9, wherein said significant portion is at least 10% by length of the total length of fluid channels through said structure.

11. The device of claim 4, said structure comprising a distribution of sizes of said fluid channels where a significant proportion of said fluid channels are large fluid channels and a significant proportion of said fluid channels are small fluid channels.

12. The device of claim 4, said structure comprising a distinct population of large fluid channels and a distinct population of small fluid channels, where the average cross-sectional area of said population of small fluid channels is less than the average cross-sectional area of said population of large fluid channels.

13. The device of claim 4, said structure having an index of refraction of not more than about 1.4.

14. The device of claim 4, wherein said structure is of a substantially rigid material.

15. The device of claim 4, said structure being substantially a solid.

16. The device of claim 4, said structure comprising a polymer.

17. The device of claim 4, said structure comprising a fluorinated hydrocarbon polymer.

18. The device of claim 4, said structure comprising an aggregate of particles of a material having an index of refraction substantially similar to that of water.

19. The device of claim 4, said structure comprising a porous block of a material having an index of refraction substantially similar to that of water.

20. The device of claim 19, said structure comprising a porous monolithic block of a material having an index of refraction substantially similar to that of water.

21. The device of claim 4, said structure comprising at least one sheet a material having an index of refraction substantially similar to that of water.

22. The device of claim 4, wherein said at least one sheet is porous.

23. The device of claim 4, said structure comprising a stack of discrete layers of a material having an index of refraction substantially similar to that of water.

24. The device of claim 4, said structure comprising at least one filament of a material having an index of refraction substantially similar to that of water.

25. The device of claim 4, said structure configured to fit inside a vessel for the study of cells.

26. The device of claim 4, further comprising a porous container in which said structure is contained.

27. The device of claim 4, further comprising a vessel in which said structure is contained, said vessel configured to hold medium.

28. The device of claim 26, wherein said structure is fixedly associated with said vessel.

29. The device of claim 26, wherein said structure is removably associated with said vessel.

30. The device of claim 26, further comprising an aqueous solution contained in said vessel.

31. The device of claim 29, further comprising a lid associated with said vessel configured to prevent loss of said aqueous solution from said vessel.

32. A method of making a structure of a device of claim 4, comprising:
a) providing a plurality of porous sheets of material having an index of refraction similar to that of water; and
b) stacking said porous sheets
thereby making the structure.

33. A method of making a structure of a device of claim 4, comprising:
a) providing at least one filament of a material having an index of refraction similar to that of water; and
b) convoluting said at least one filament,
thereby making the structure.

34. A method of making a structure of a device of claim 4, comprising:
a) providing at least one sheet of a material having an index of refraction similar to that of water; and
b) arranging said at least one sheet so as to make the structure.

35. The method of claim 34, wherein said arranging comprises at least one action selected from the group consisting of folding, crumpling and coiling.

36. A method of making a structure of a device of claim 4, comprising:
a) providing a plurality of particles of material having an index of refraction similar to that of water; and
b) aggregating said particles so as to provide a porous block
thereby making the structure.

37. A method for studying cells, comprising:

a) seeding a structure of a device of claim 1 with at least one viable cell;

b) immersing said structure in a medium; and

c) observing development of said cells inside said structure.

38. The method of claim 36, further comprising:

d) adding an active entity to said medium; and

e) observing an effect on said at least one cell of said adding of said active entity.

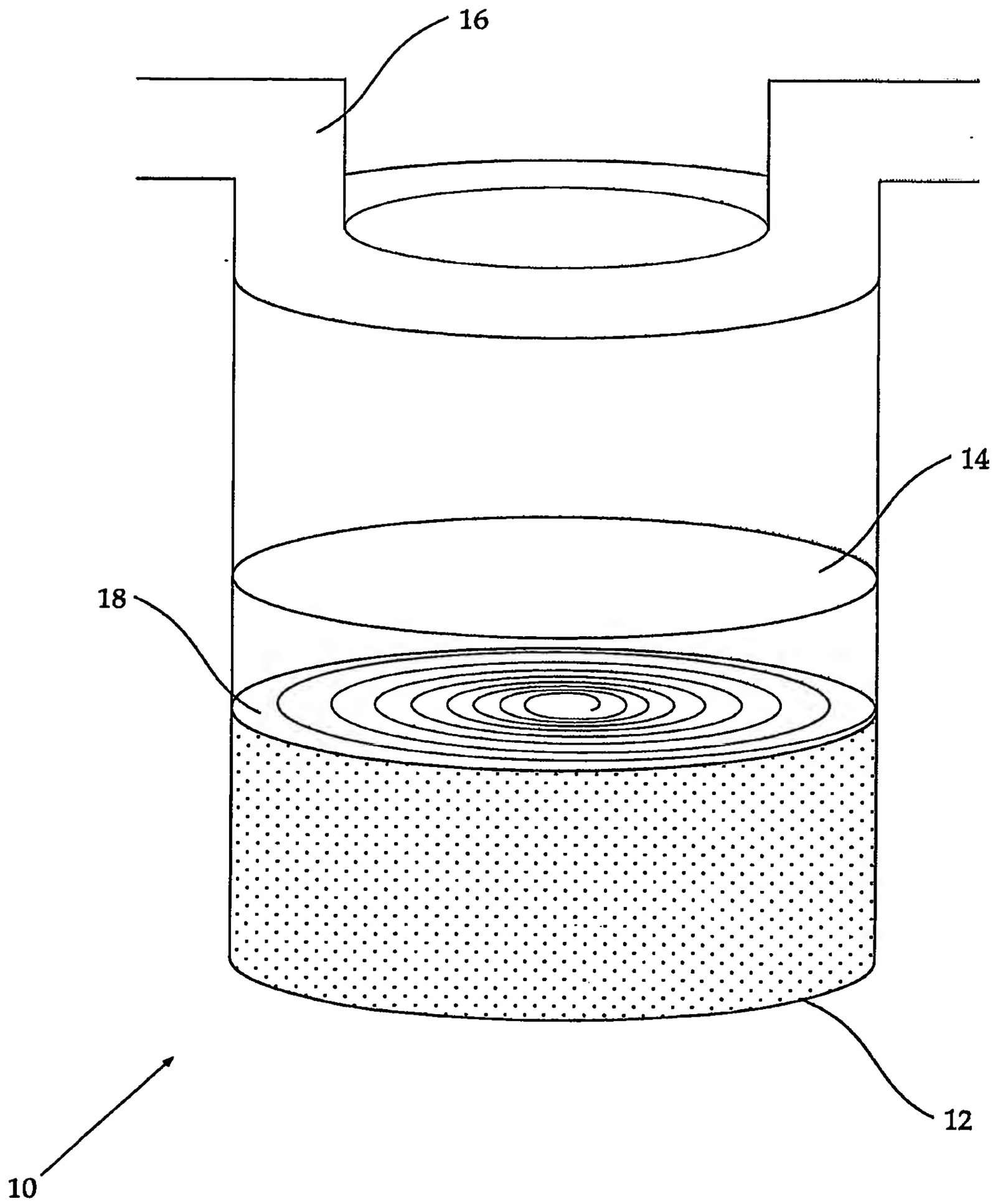

FIG. 1

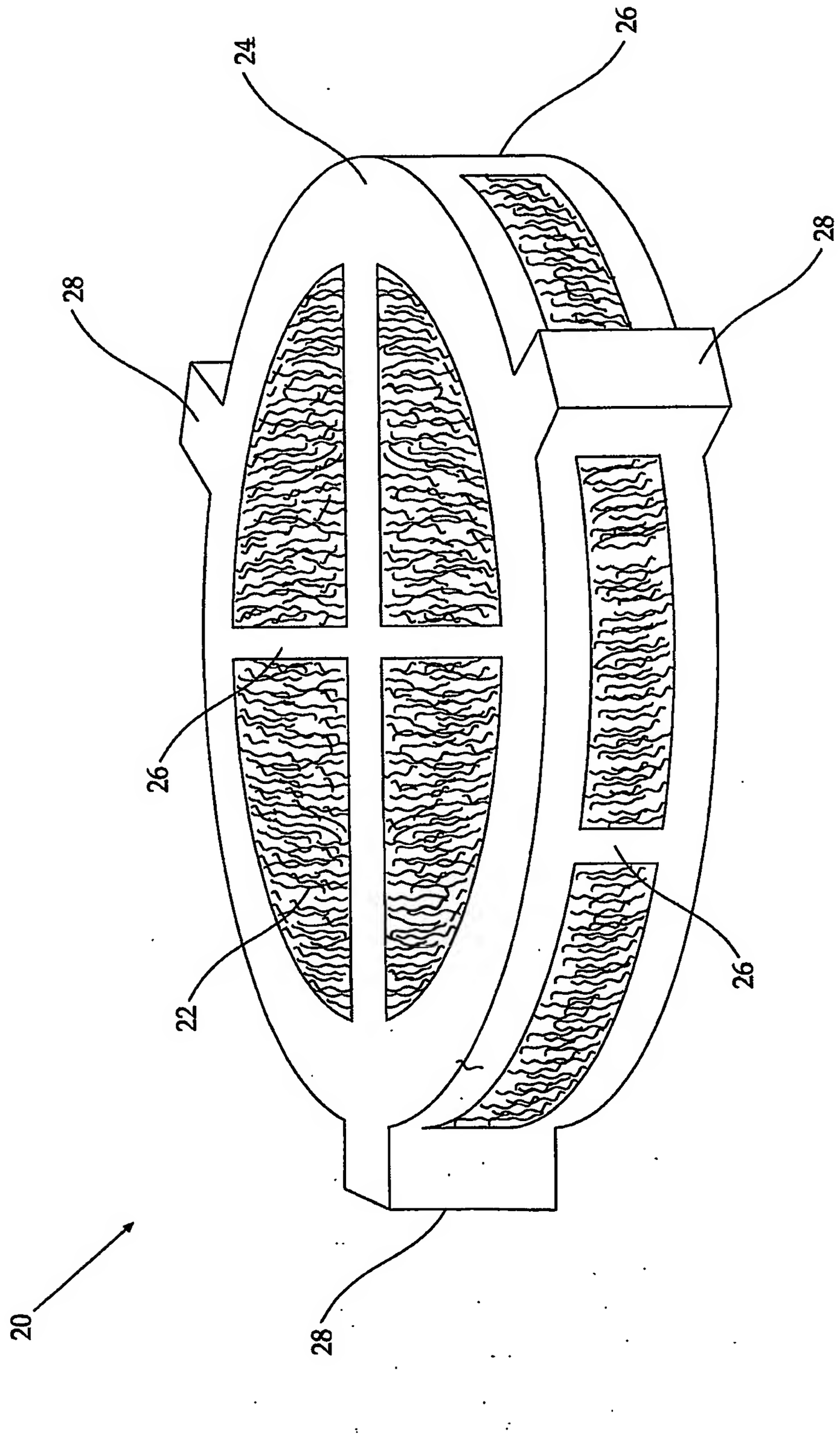

FIG. 2

FIG. 3A

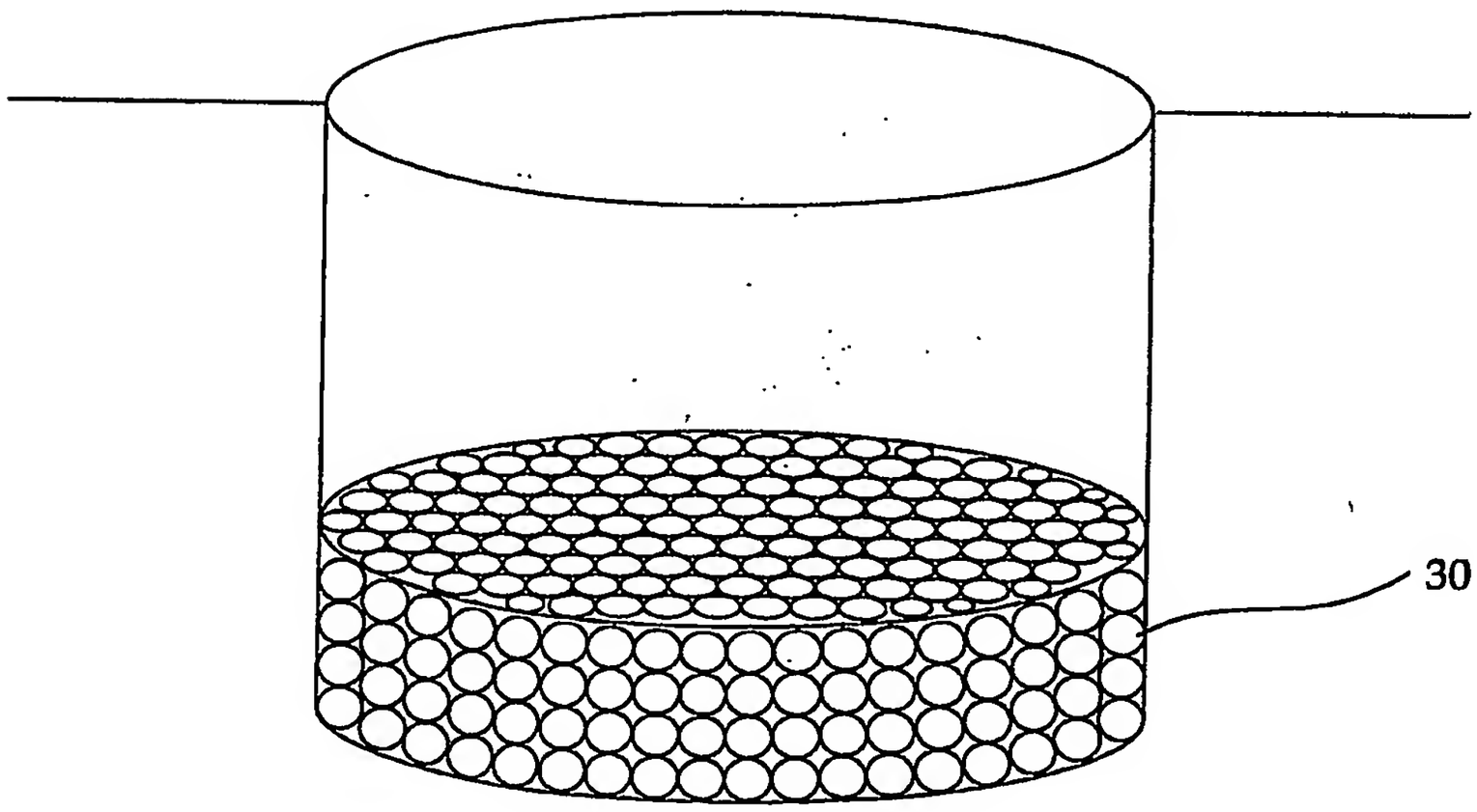

FIG. 3B

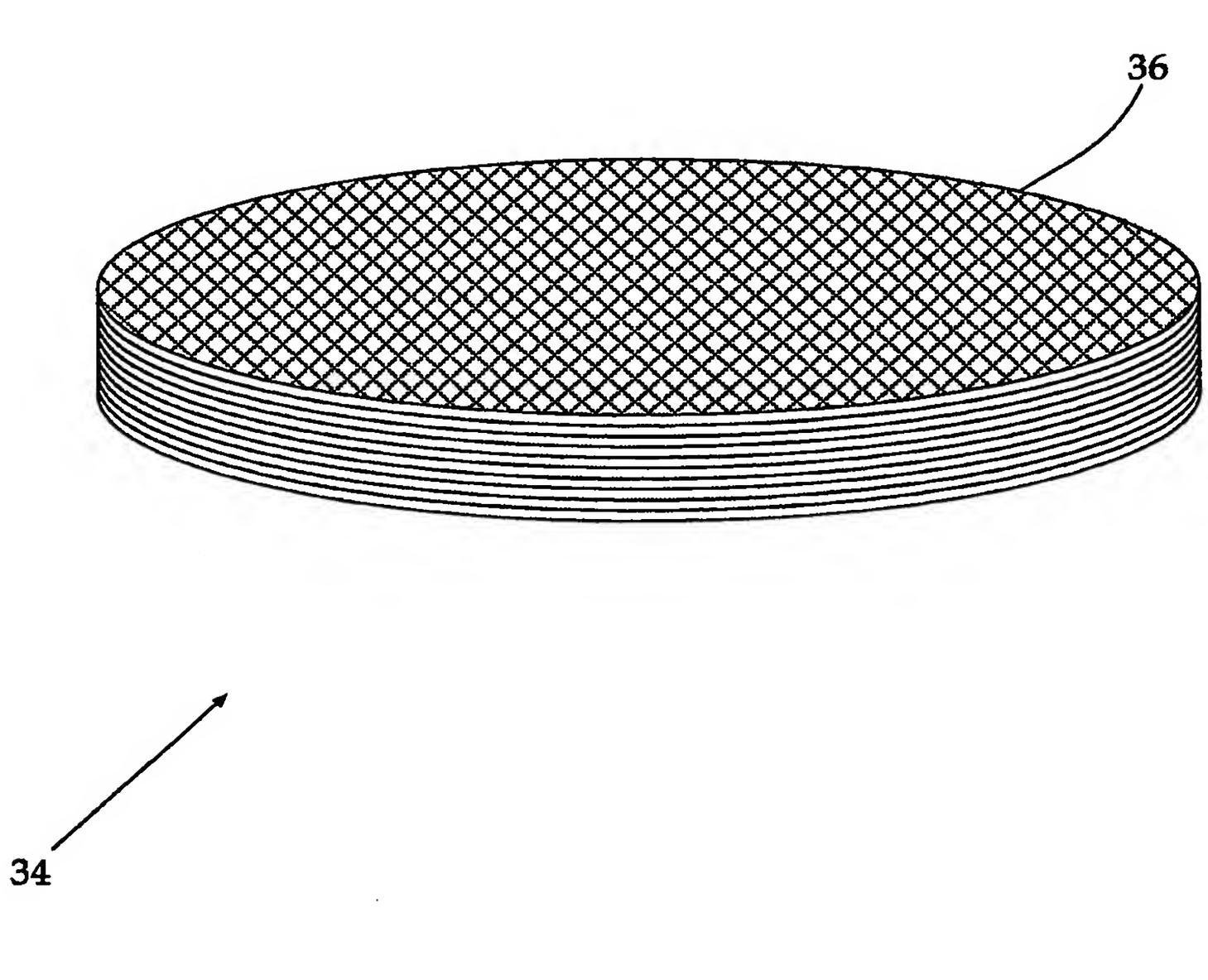

FIG. 4